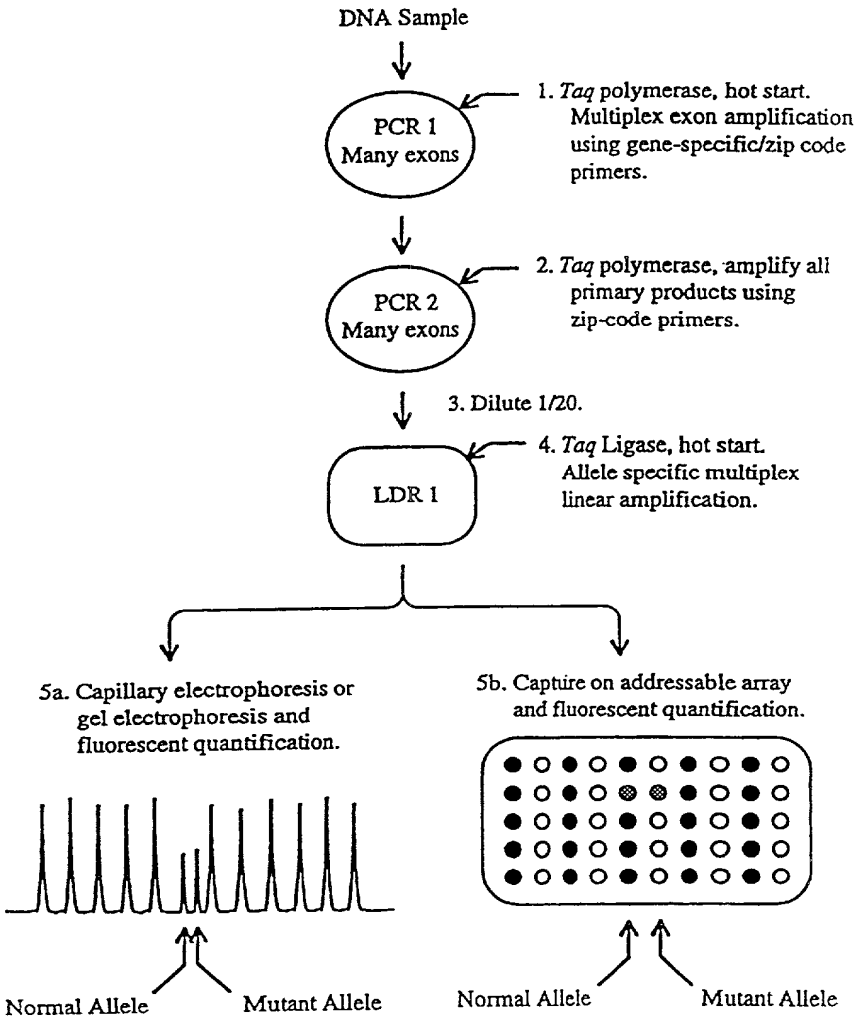

DNA Sample
PCR 1
Many exons
1. Taq polymerase, hot start. Multiplex exon amplification using gene-specific/zip code primers.
PCR 2
Many exons
2. Taq polymerase, amplify all primary products using zip-code primers.
3. Dilute 1/20.
LDR 1
4. Taq Ligase, hot start. Allele specific multiplex linear amplification.
5a. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
5b. Capture on addressable array and fluorescent quantification.
Normal Allele
Mutant Allele
Normal Allele
Mutant Allele

FIG. 1

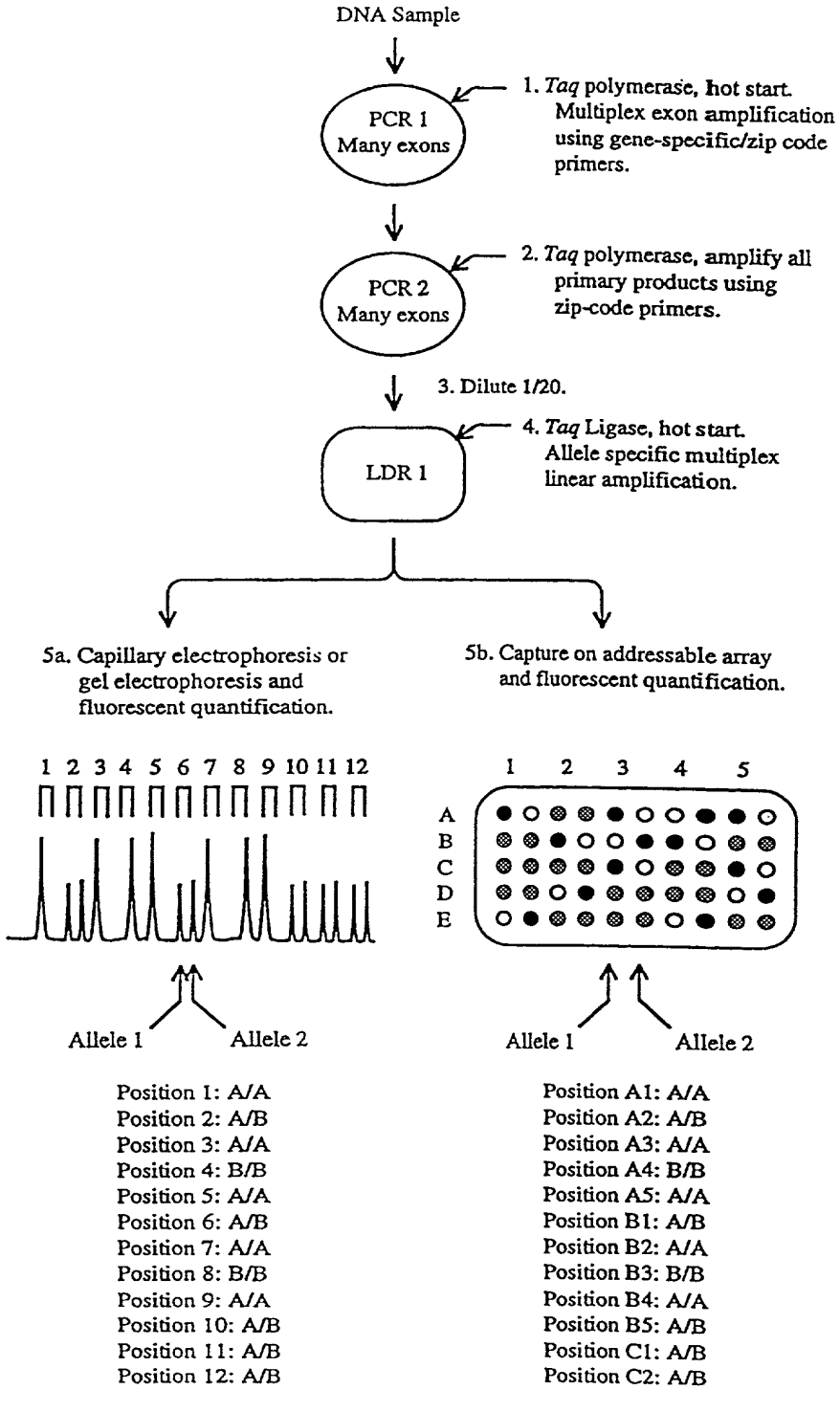

DNA Sample
PCR 1
Many exons
1. Taq polymerase, hot start. Multiplex exon amplification using gene-specific/zip code primers.
PCR 2
Many exons
2. Taq polymerase, amplify all primary products using zip-code primers.
3. Dilute 1/20.
LDR 1
4. Taq Ligase, hot start. Allele specific multiplex linear amplification.
5a. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
5b. Capture on addressable array and fluorescent quantification.
1 2 3 4 5 6 7 8 9 10 11 12
1 2 3 4 5
A
B
C
D
E
Allele 1
Allele 2
Allele 1
Allele 2
Position 1: A/A
Position 2: A/B
Position 3: A/A
Position 4: B/B
Position 5: A/A
Position 6: A/B
Position 7: A/A
Position 8: B/B
Position 9: A/A
Position 10: A/B
Position 11: A/B
Position 12: A/B
Position A1: A/A
Position A2: A/B
Position A3: A/A
Position A4: B/B
Position A5: A/A
Position B1: A/B
Position B2: A/A
Position B3: B/B
Position B4: A/A
Position B5: A/B
Position C1: A/B
Position C2: A/B

FIG. 2

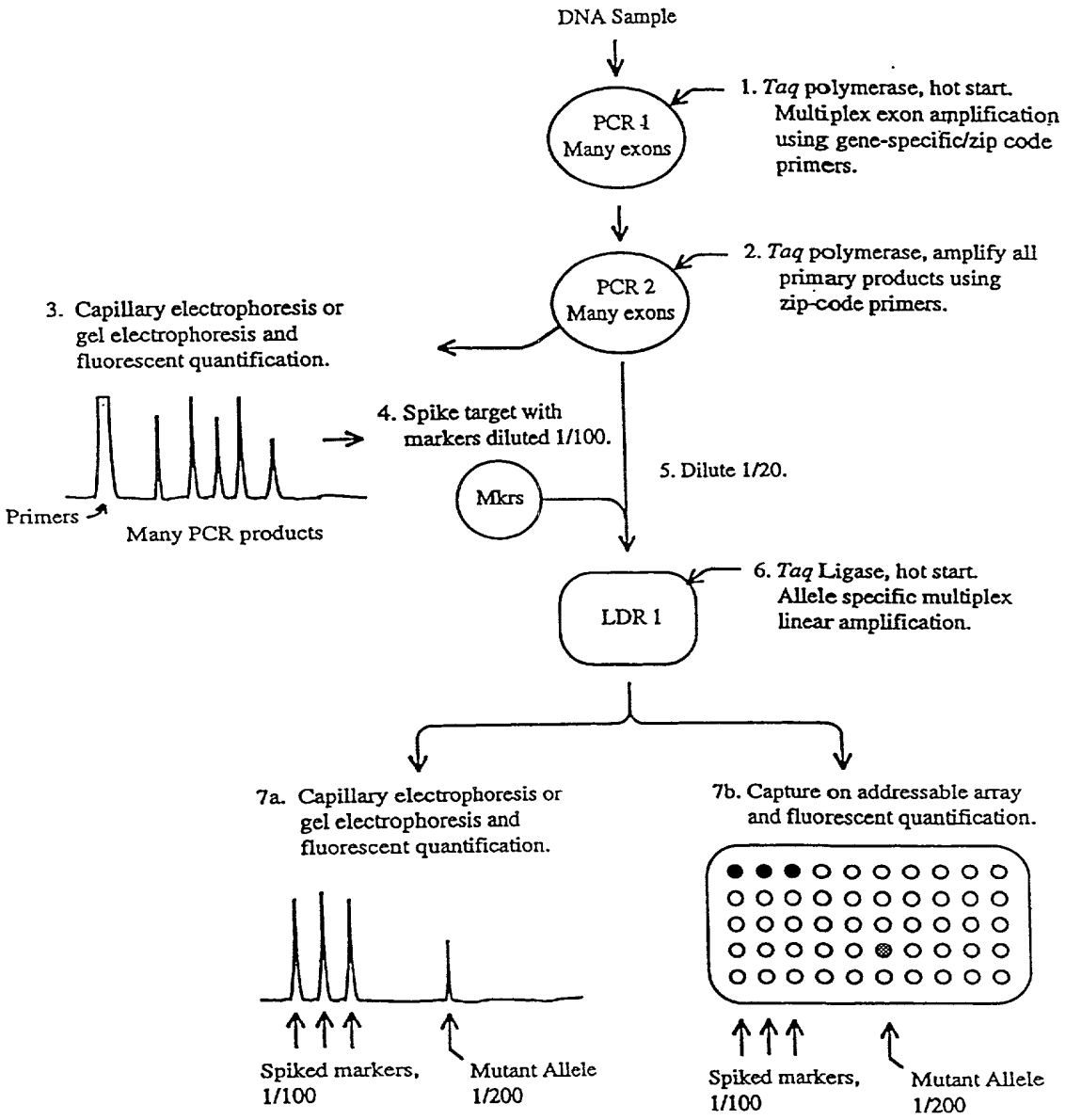

DNA Sample
PCR 1
Many exons
1. *Taq* polymerase, hot start. Multiplex exon amplification using gene-specific/zip code primers.
PCR 2
Many exons
2. *Taq* polymerase, amplify all primary products using zip-code primers.
3. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
Primers
Many PCR products
4. Spike target with markers diluted 1/100.
Mkrs
5. Dilute 1/20.
LDR 1
6. *Taq* Ligase, hot start. Allele specific multiplex linear amplification.
7a. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
Spiked markers, 1/100
Mutant Allele 1/200
7b. Capture on addressable array and fluorescent quantification.
Spiked markers, 1/100
Mutant Allele 1/200

FIG. 3

1. PCR amplify regions containing allelic variations using gene-specific/zip code primers, dNTPs and *Taq* polymerase. ◆
2. PCR amplify all primary products using zip code primers, dNTPs and *Taq* polymerase.
3. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.
4. Separate fluorescent products on a DNA sequencer and quantify each allele.

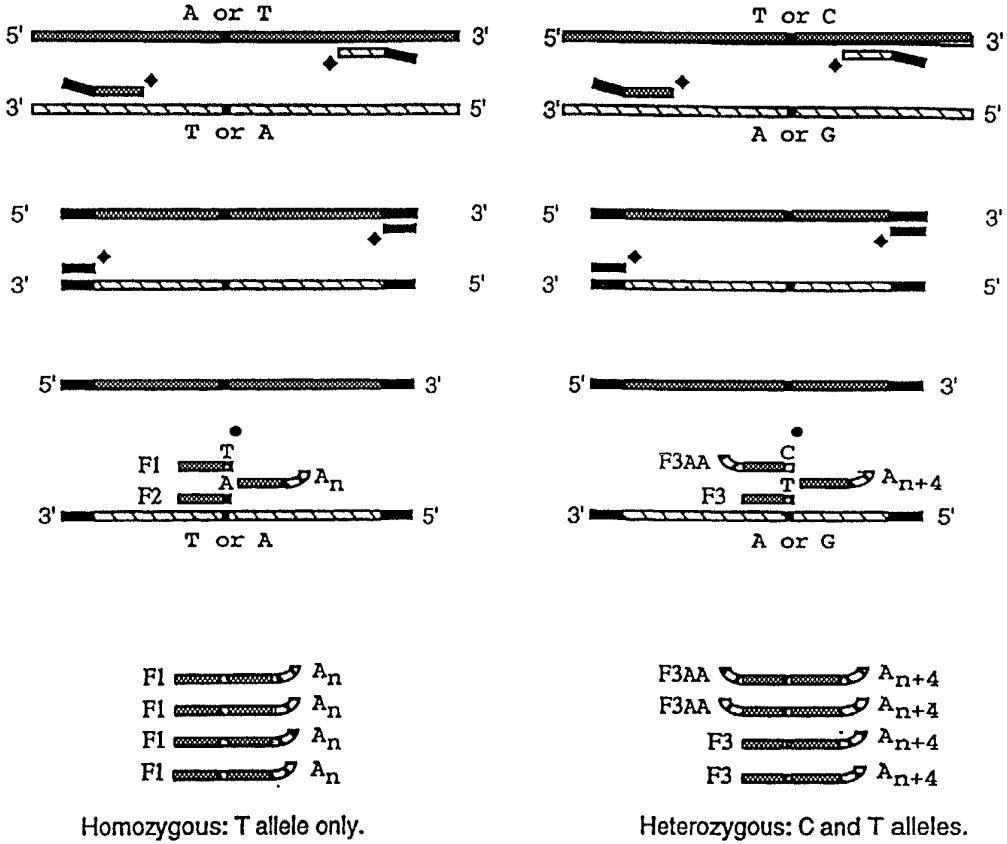
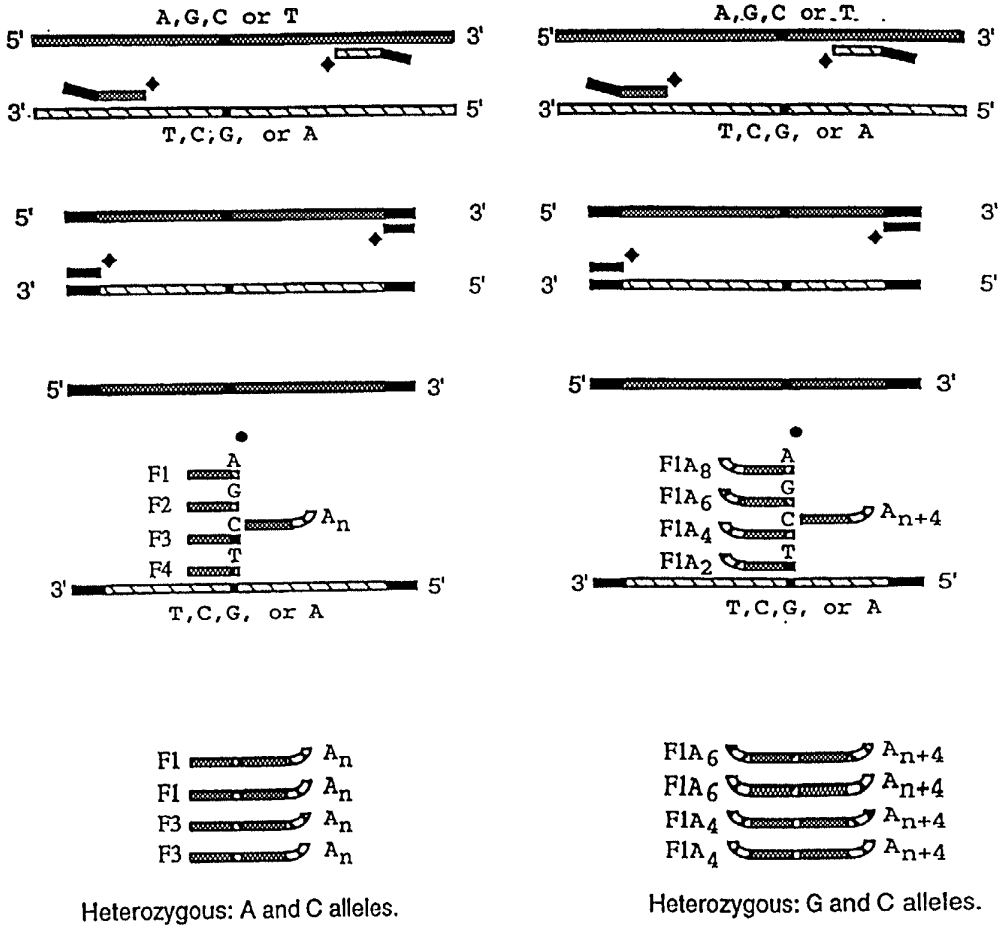


FIG. 4

PCR/ PCR/ LDR

1. PCR amplify regions containing allelic variations using gene-specific/zip code primers, dNTPs and *Taq* polymerase. ◆
2. PCR amplify all primary products using zip code primers, dNTPs and *Taq* polymerase.
3. Perform LDR using allele-specific LDR primers and thermostable ligase. ● Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.
4. Separate fluorescent products on a DNA sequencer and quantify each allele.



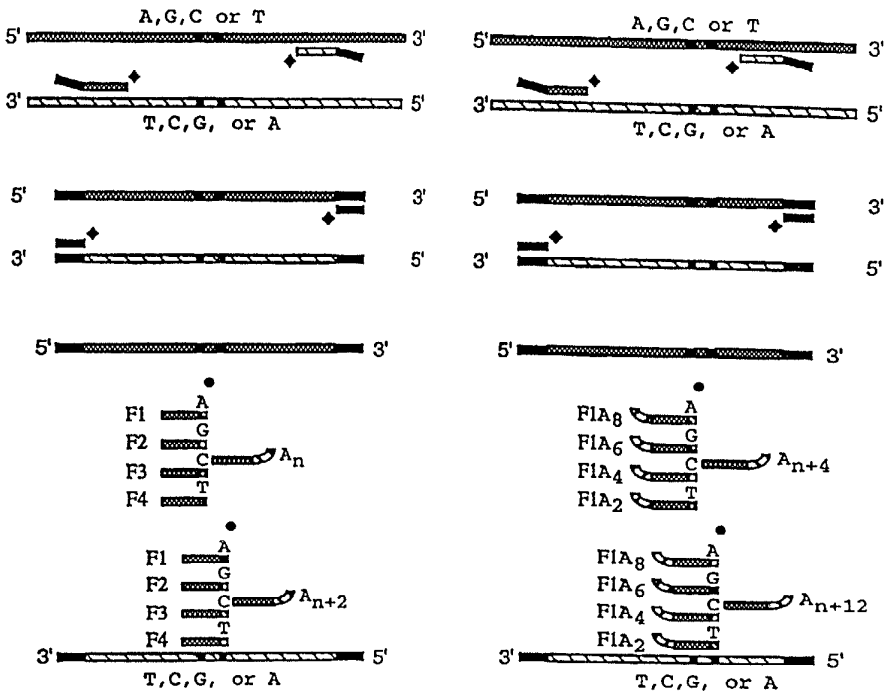
Heterozygous: A and C alleles.

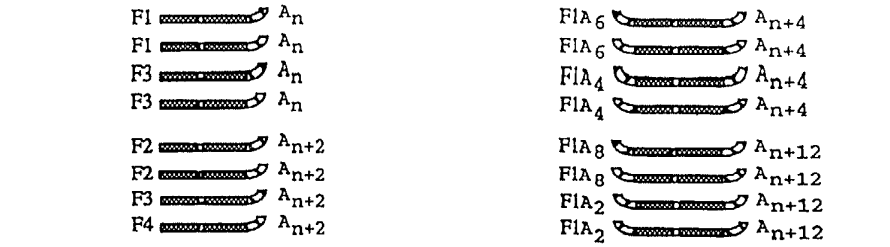
Heterozygous: G and C alleles.

FIG. 5

PCR/ PCR/ LDR : Nearby alleles

1. PCR amplify regions containing allelic variations using gene-specific/zip code primers, dNTPs and *Taq* polymerase. ◆
2. PCR amplify all primary products using zip code primers, dNTPs and *Taq* polymerase.
3. Perform LDR using allele-specific LDR primers and ● thermostable ligase. Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.
4. Separate fluorescent products on a DNA sequencer and quantify each allele.





1st Position.
Heterozygous: A and C alleles.
2nd Position.
Heterozygous: G,C and T alleles.

1st Position.
Heterozygous: G and C alleles.
2nd Position.
Heterozygous: A and T alleles.

FIG. 6

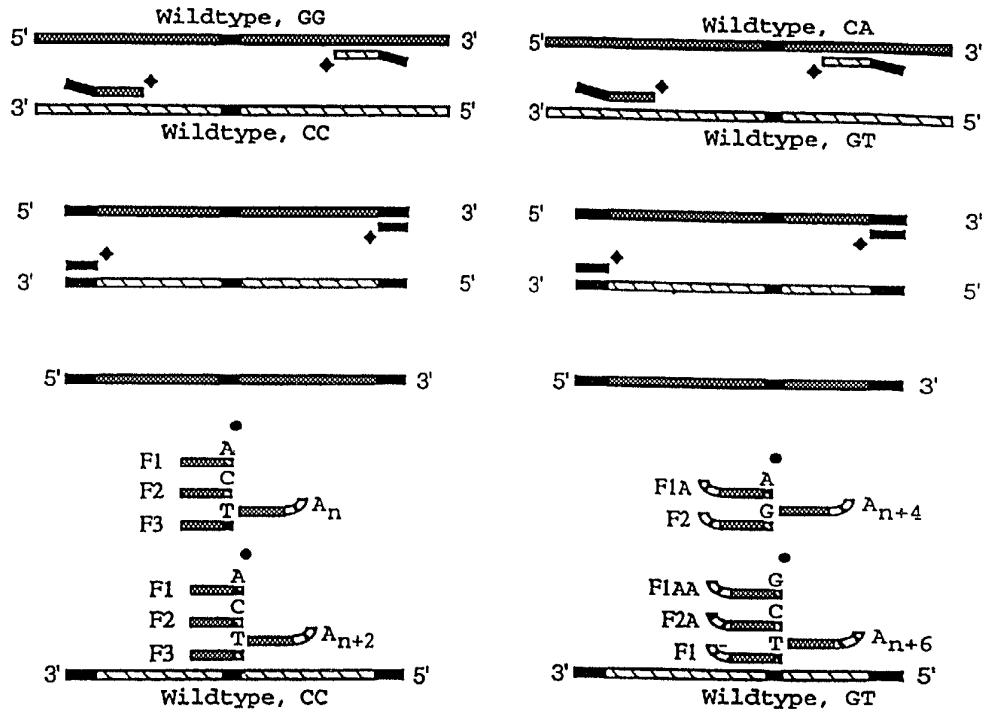
PCR/ PCR/ LDR : Adjacent alleles, cancer detection

1. PCR amplify regions containing allelic variations using gene-specific/zip code primers, dNTPs and *Taq* polymerase. ◆

2. PCR amplify all primary products using zip code primers, dNTPs and *Taq* polymerase.

3. Perform LDR using allele-specific LDR primers and ● thermostable ligase. Allele-specific oligonucleotides ligate to common oligonucleotides only when there is perfect complementarity at the junction.

4. Separate fluorescent products on a DNA sequencer and quantify each allele.



Gly to Asp mutation

Gln to Glu mutation

FIG. 7

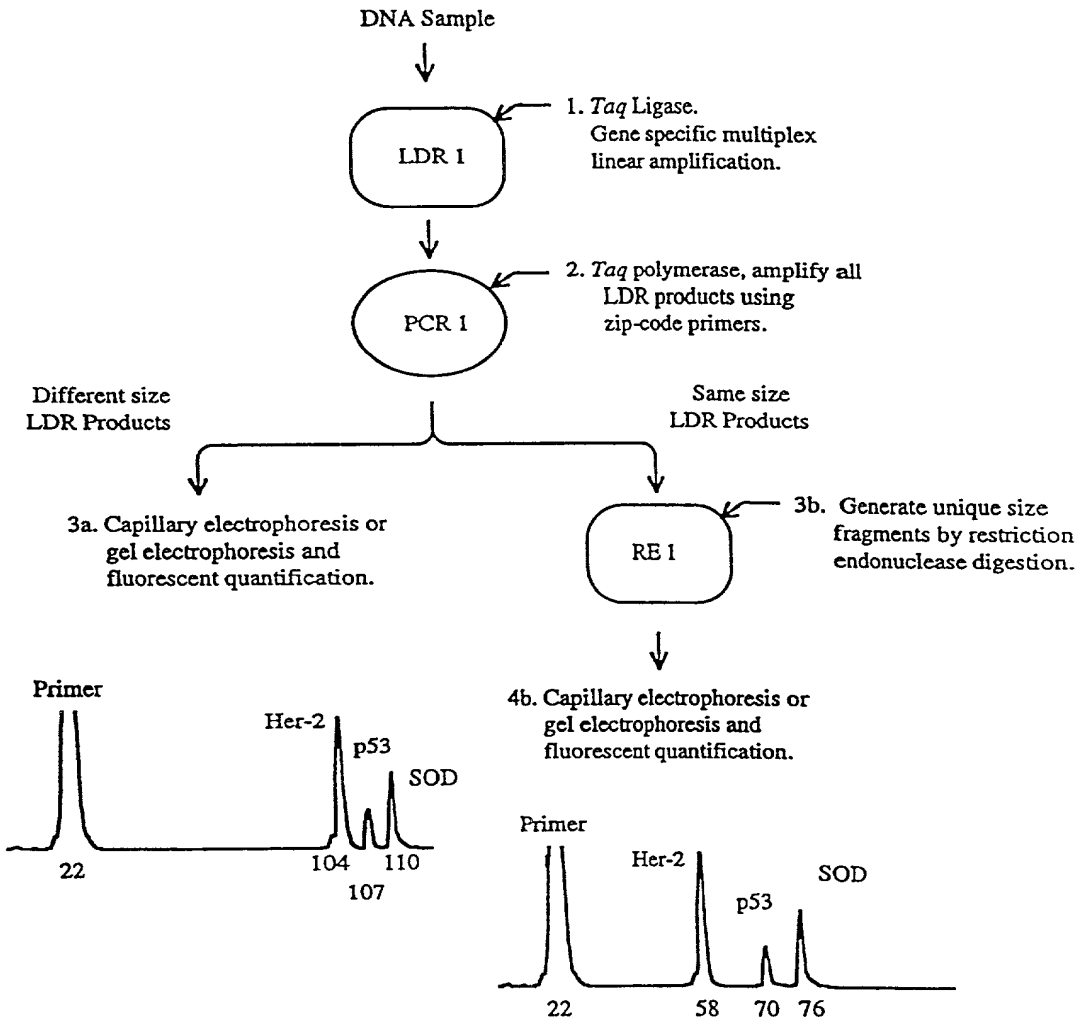

DNA Sample
LDR 1
1. Taq Ligase.
Gene specific multiplex
linear amplification.
PCR 1
2. Taq polymerase, amplify all
LDR products using
zip-code primers.
Different size
LDR Products
Same size
LDR Products
3a. Capillary electrophoresis or
gel electrophoresis and
fluorescent quantification.
RE 1
3b. Generate unique size
fragments by restriction
endonuclease digestion.
4b. Capillary electrophoresis or
gel electrophoresis and
fluorescent quantification.
Primer
Her-2
p53
SOD
22
104
107
110
Primer
Her-2
p53
SOD
22
58
70
76

FIG. 8

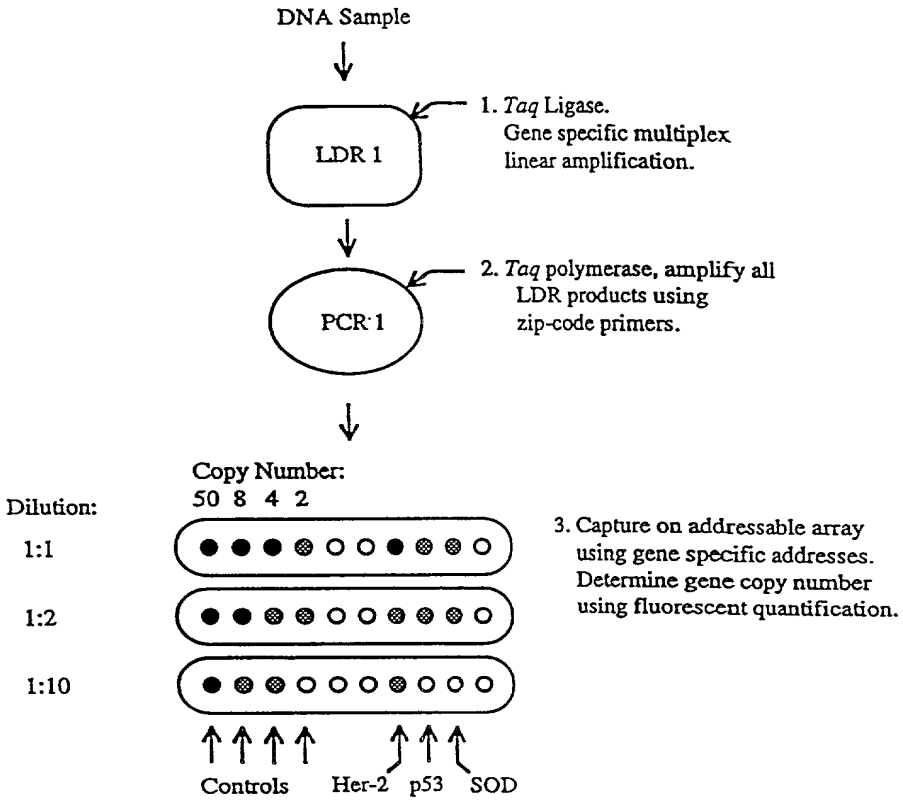

DNA Sample
LDR 1
1. *Taq* Ligase. Gene specific multiplex linear amplification.
PCR 1
2. *Taq* polymerase, amplify all LDR products using zip-code primers.
Copy Number:
50 8 4 2
Dilution:
1:1
1:2
1:10
3. Capture on addressable array using gene specific addresses. Determine gene copy number using fluorescent quantification.
Controls
Her-2
p53
SOD

FIG. 9

LDR / PCR: Multiplex detection of gene amplifications and deletions

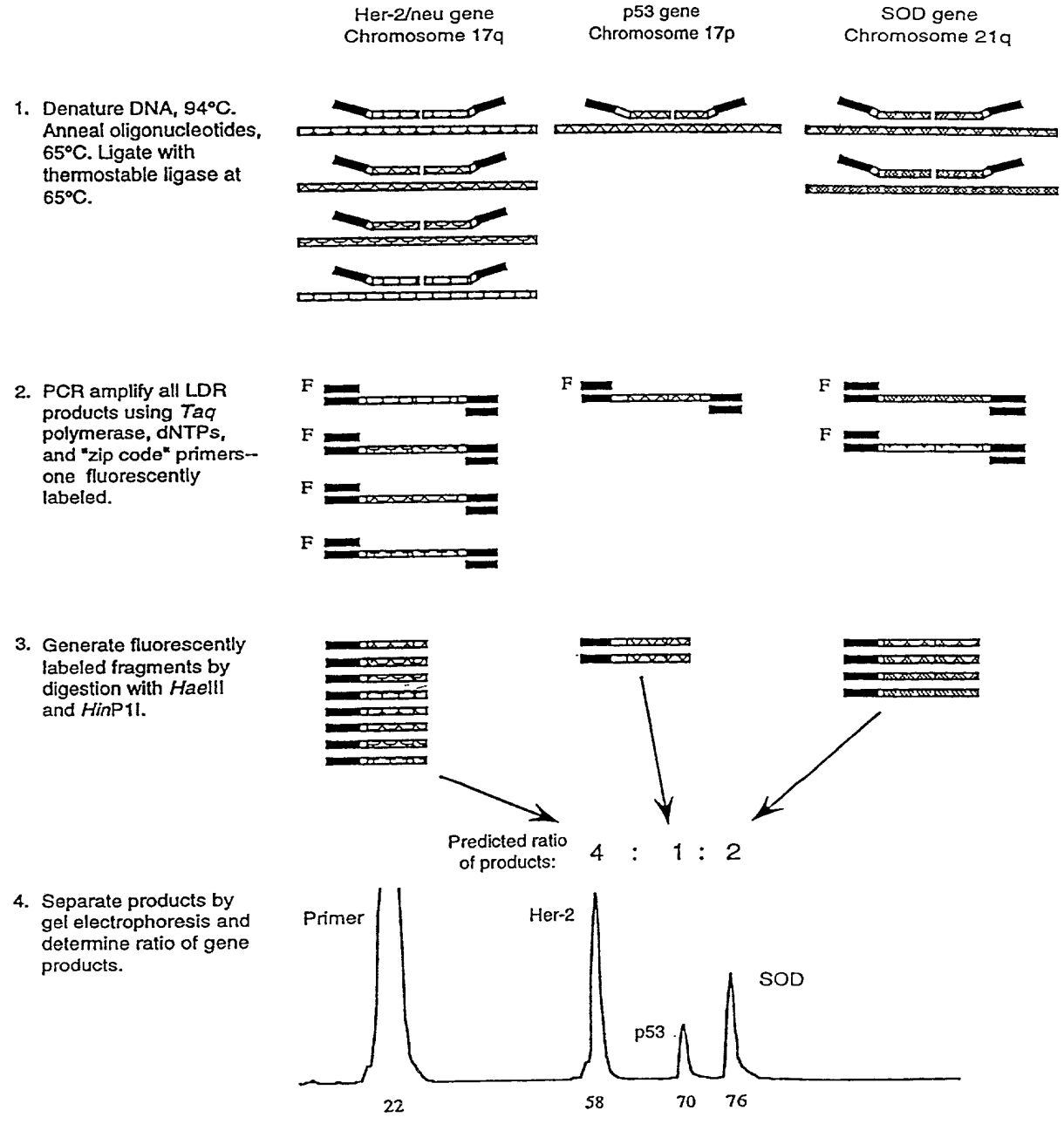


FIG. 10

Allele specific LDR / PCR Problem

1. Denature DNA, 94°C. Anneal allele-specific LDR primers 65°C. Ligate with thermostable ligase at 65°C. ●

2. PCR amplify all primary products using zip code primers, dNTPs and *Taq* polymerase. ◆

3. LDR product from mutant sequence generates fluorescence PCR product. Unfortunately, PCR extension of LDR primer off original normal target also generates fluorescence PCR product.

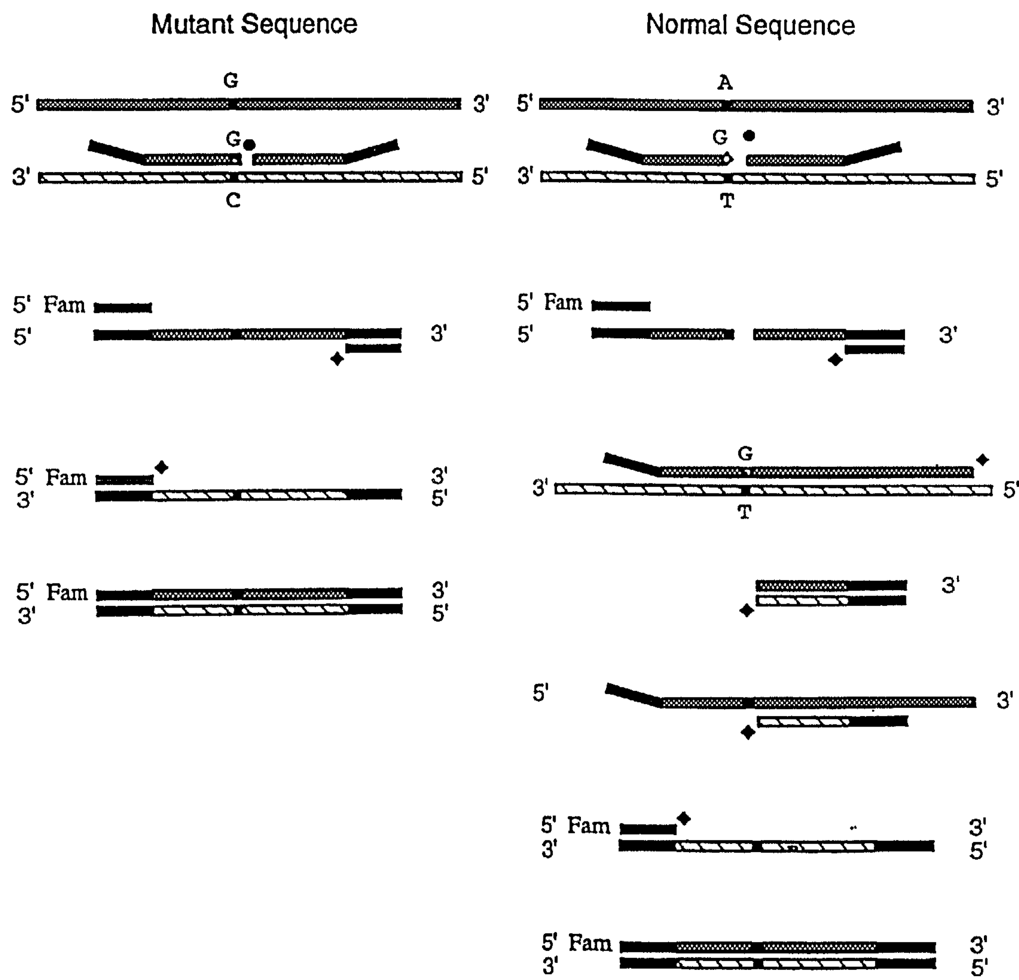


FIG. 11

Solution to allele specific LDR / PCR problem

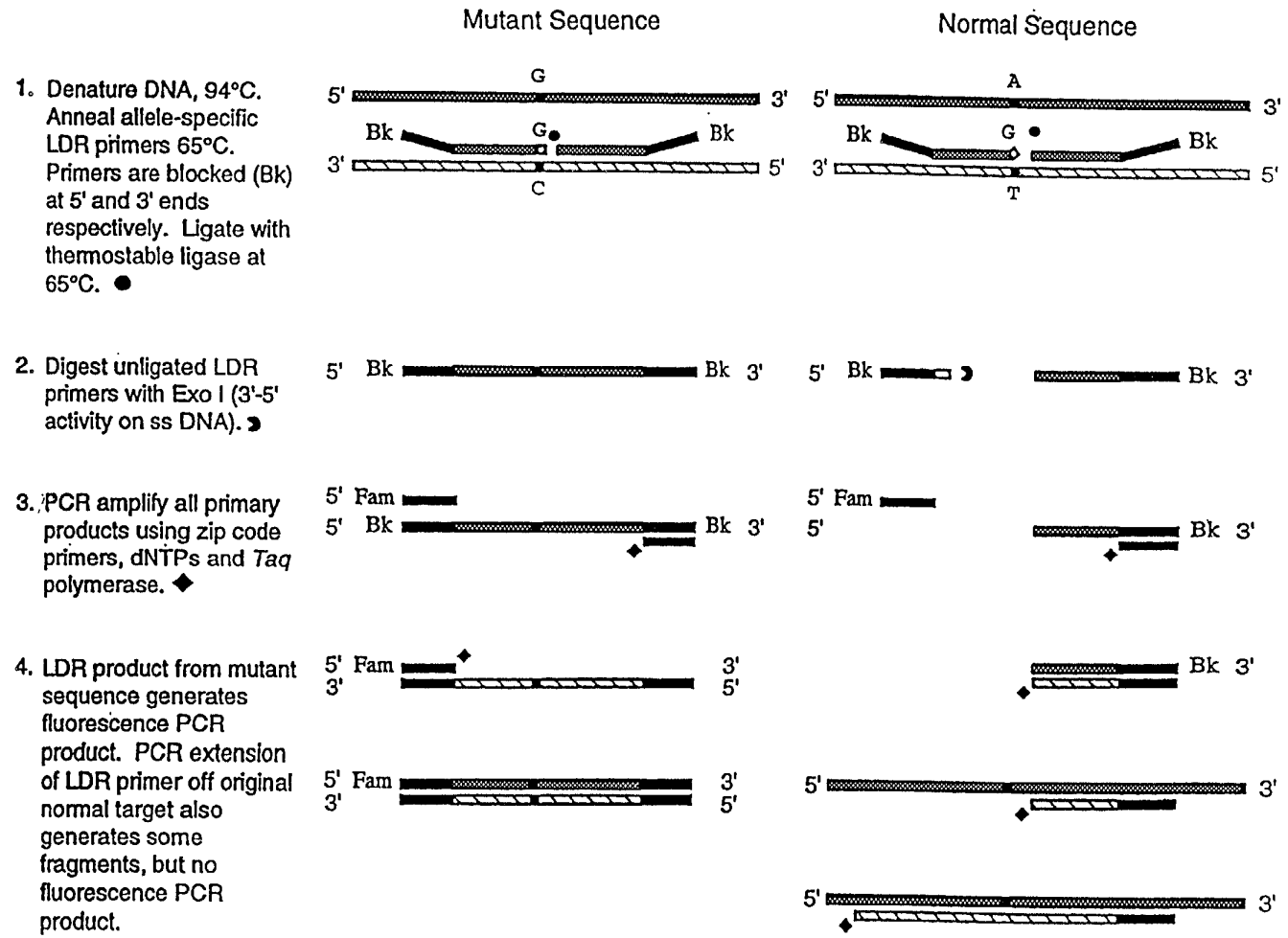


FIG. 12

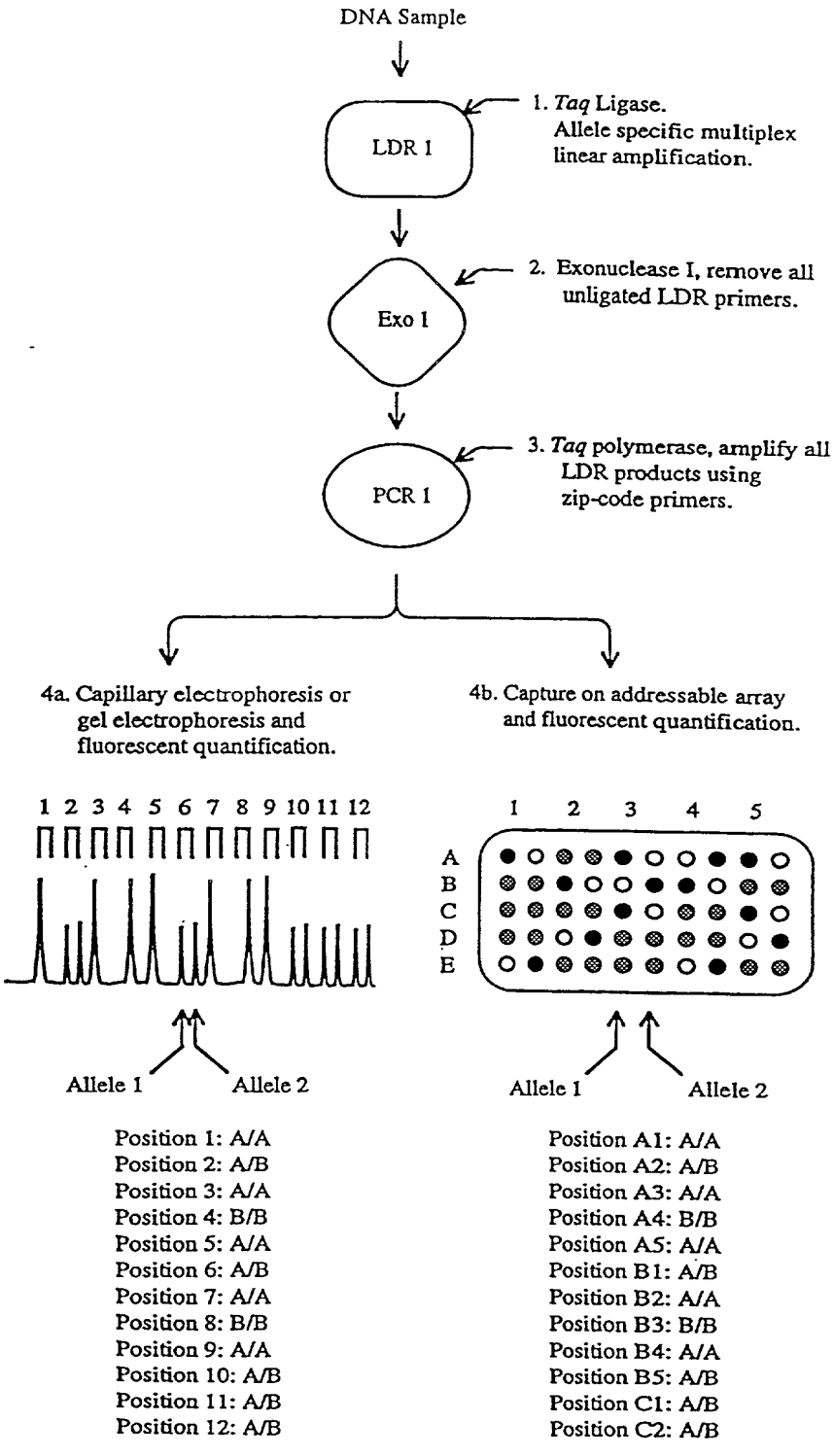

DNA Sample
LDR 1
1. Taq Ligase. Allele specific multiplex linear amplification.
Exo 1
2. Exonuclease I, remove all unligated LDR primers.
PCR 1
3. Taq polymerase, amplify all LDR products using zip-code primers.
4a. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
4b. Capture on addressable array and fluorescent quantification.
1 2 3 4 5 6 7 8 9 10 11 12
A
B
C
D
E
1 2 3 4 5
Allele 1
Allele 2
Allele 1
Allele 2
Position 1: A/A
Position 2: A/B
Position 3: A/A
Position 4: B/B
Position 5: A/A
Position 6: A/B
Position 7: A/A
Position 8: B/B
Position 9: A/A
Position 10: A/B
Position 11: A/B
Position 12: A/B
Position A1: A/A
Position A2: A/B
Position A3: A/A
Position A4: B/B
Position A5: A/A
Position B1: A/B
Position B2: A/A
Position B3: B/B
Position B4: A/A
Position B5: A/B
Position C1: A/B
Position C2: A/B

FIG. 13

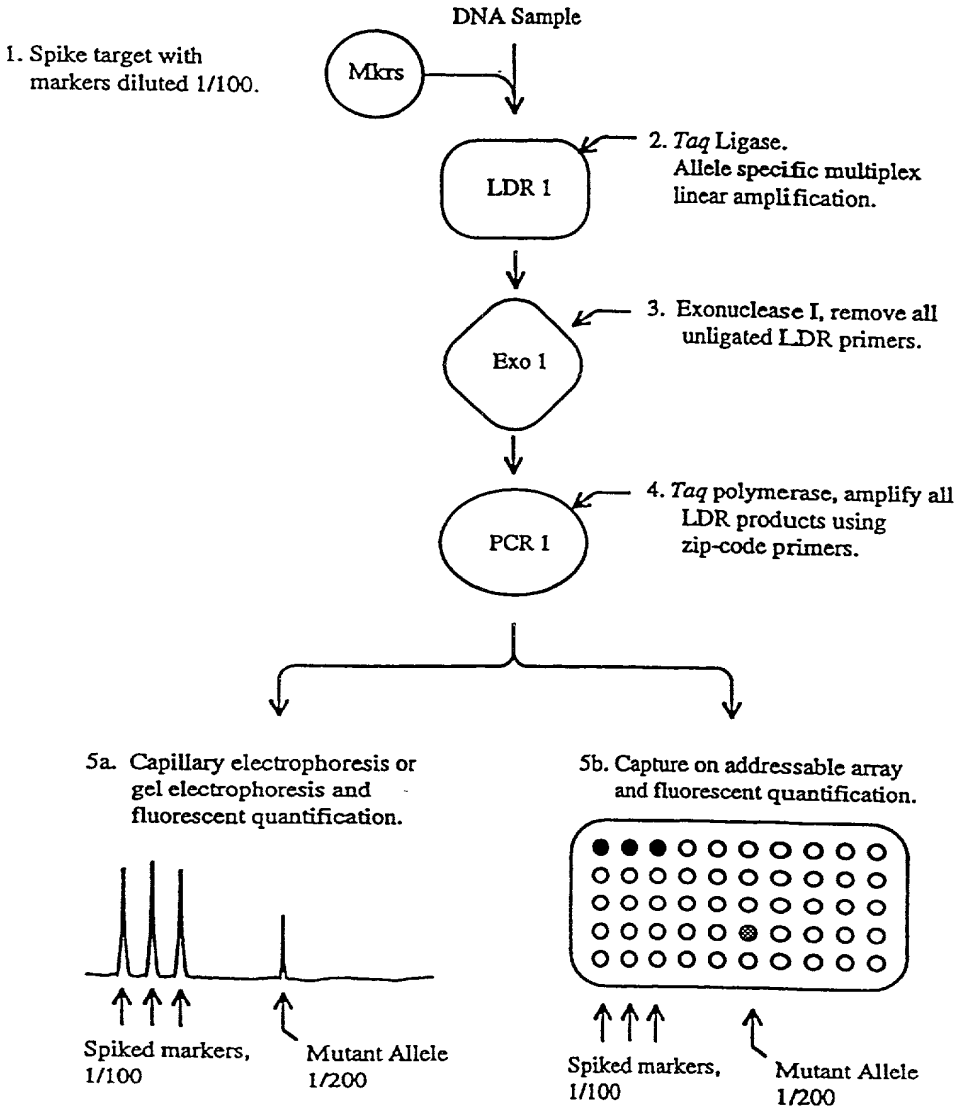

DNA Sample
1. Spike target with markers diluted 1/100.
Mkrs
LDR 1
2. Taq Ligase. Allele specific multiplex linear amplification.
Exo 1
3. Exonuclease I, remove all unligated LDR primers.
PCR 1
4. Taq polymerase, amplify all LDR products using zip-code primers.
5a. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
5b. Capture on addressable array and fluorescent quantification.
Spiked markers, 1/100
Mutant Allele 1/200
Spiked markers, 1/100
Mutant Allele 1/200

FIG. 14

Allele specific LDR / PCR for mutations or polymorphisms

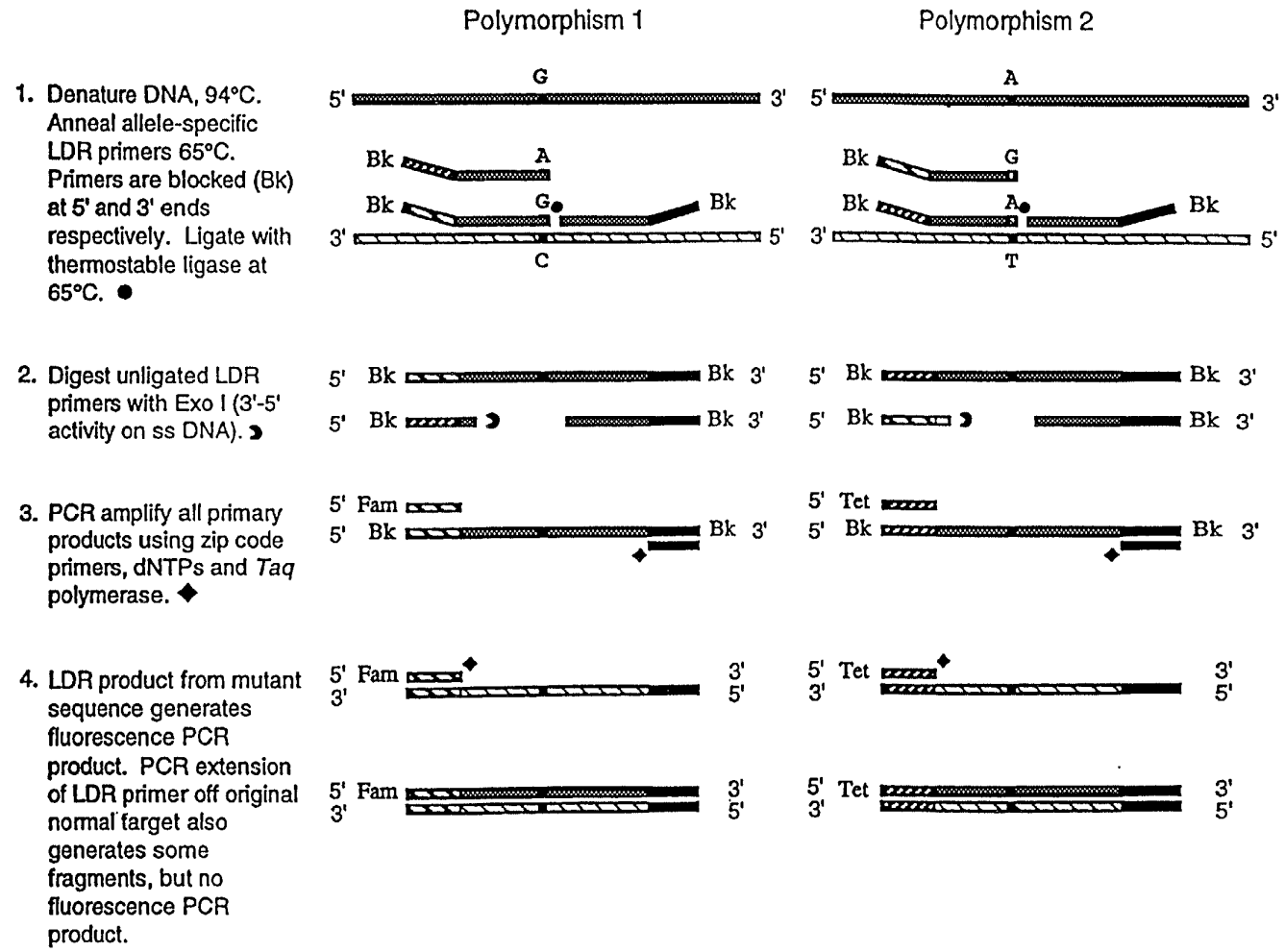


FIG. 15

LDR / PCR of mononucleotide repeats using exonuclease selection

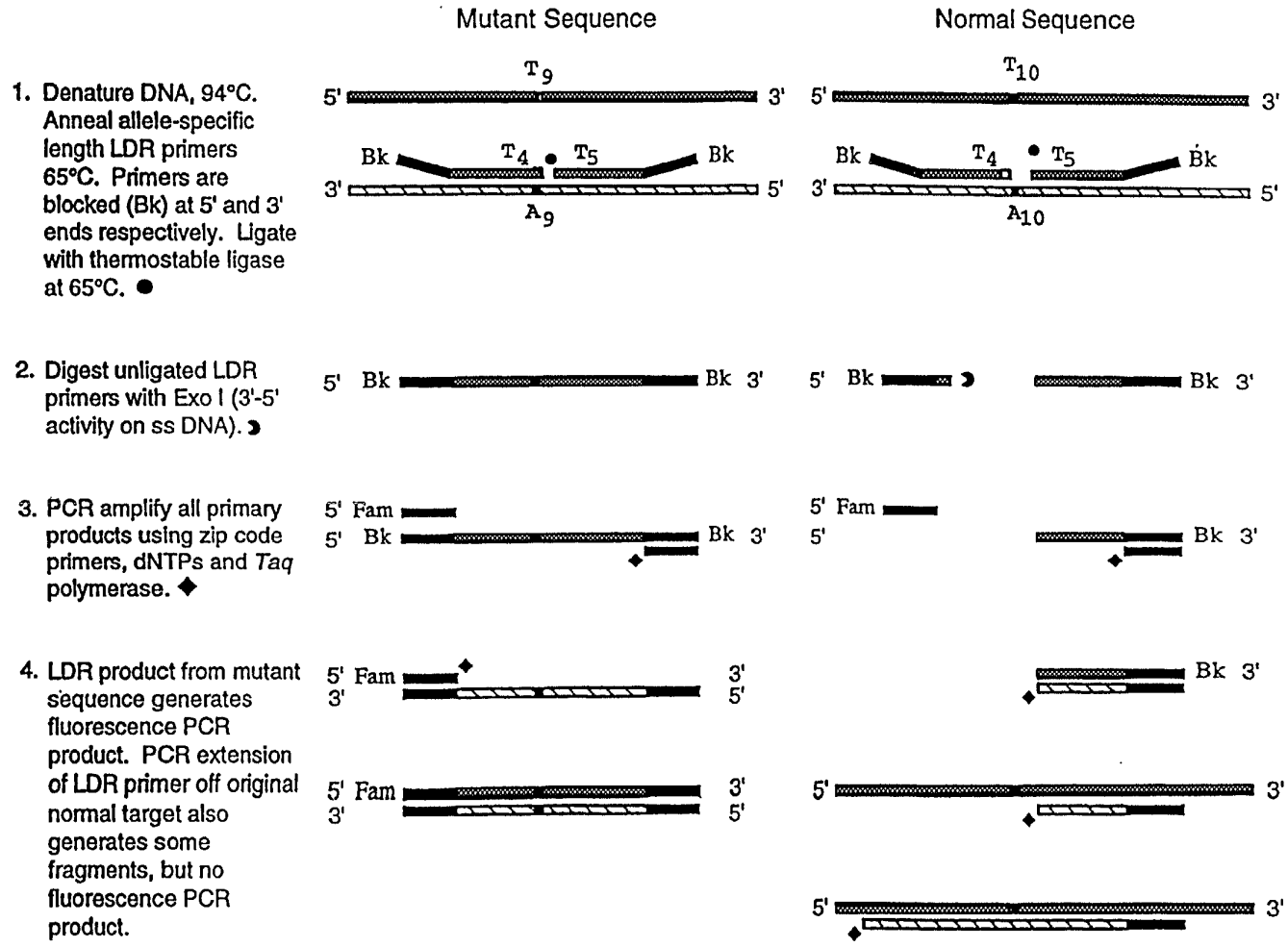


FIG. 16

LDR / PCR of mononucleotide repeat polymorphisms using exonuclease selection

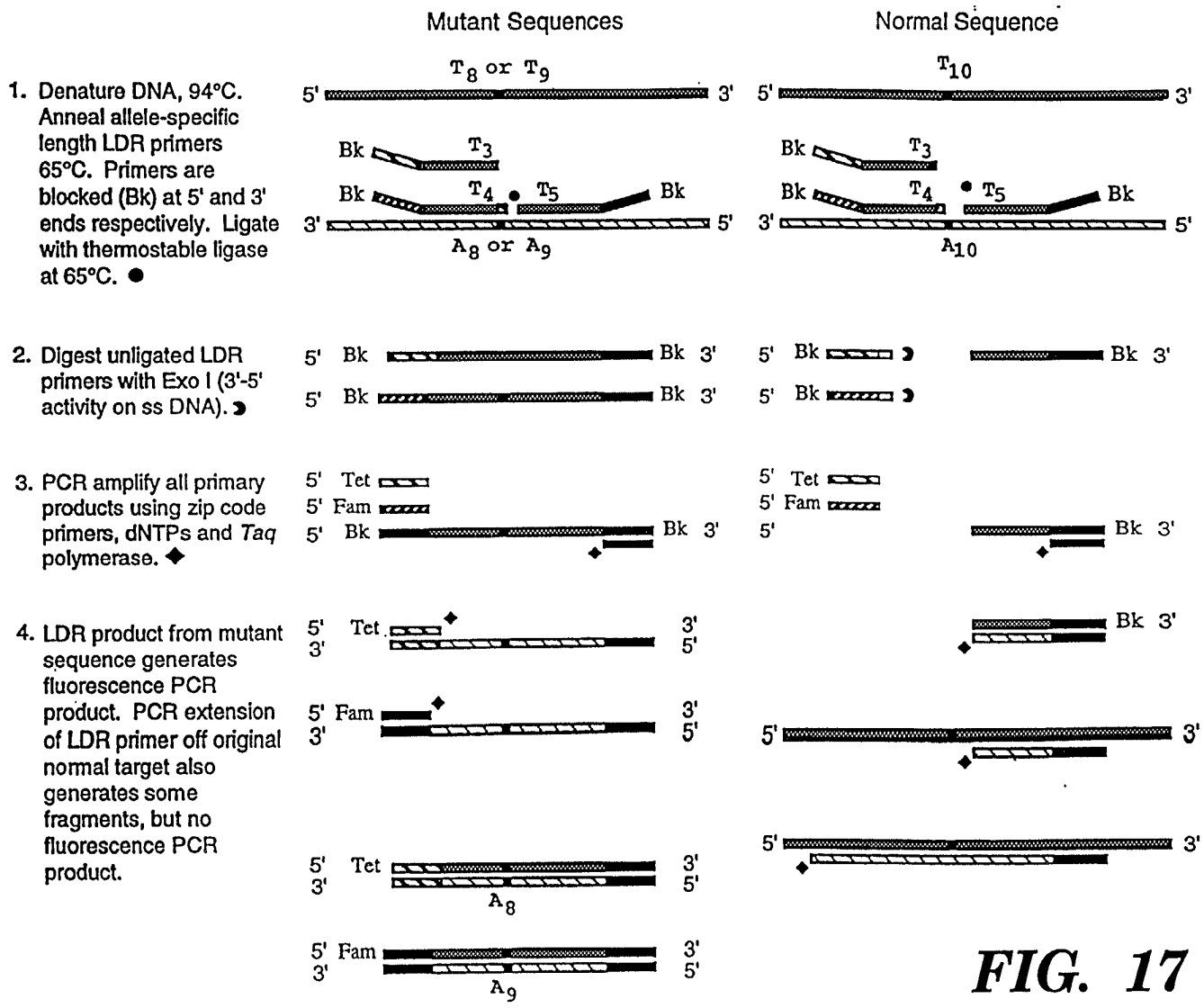


FIG. 17

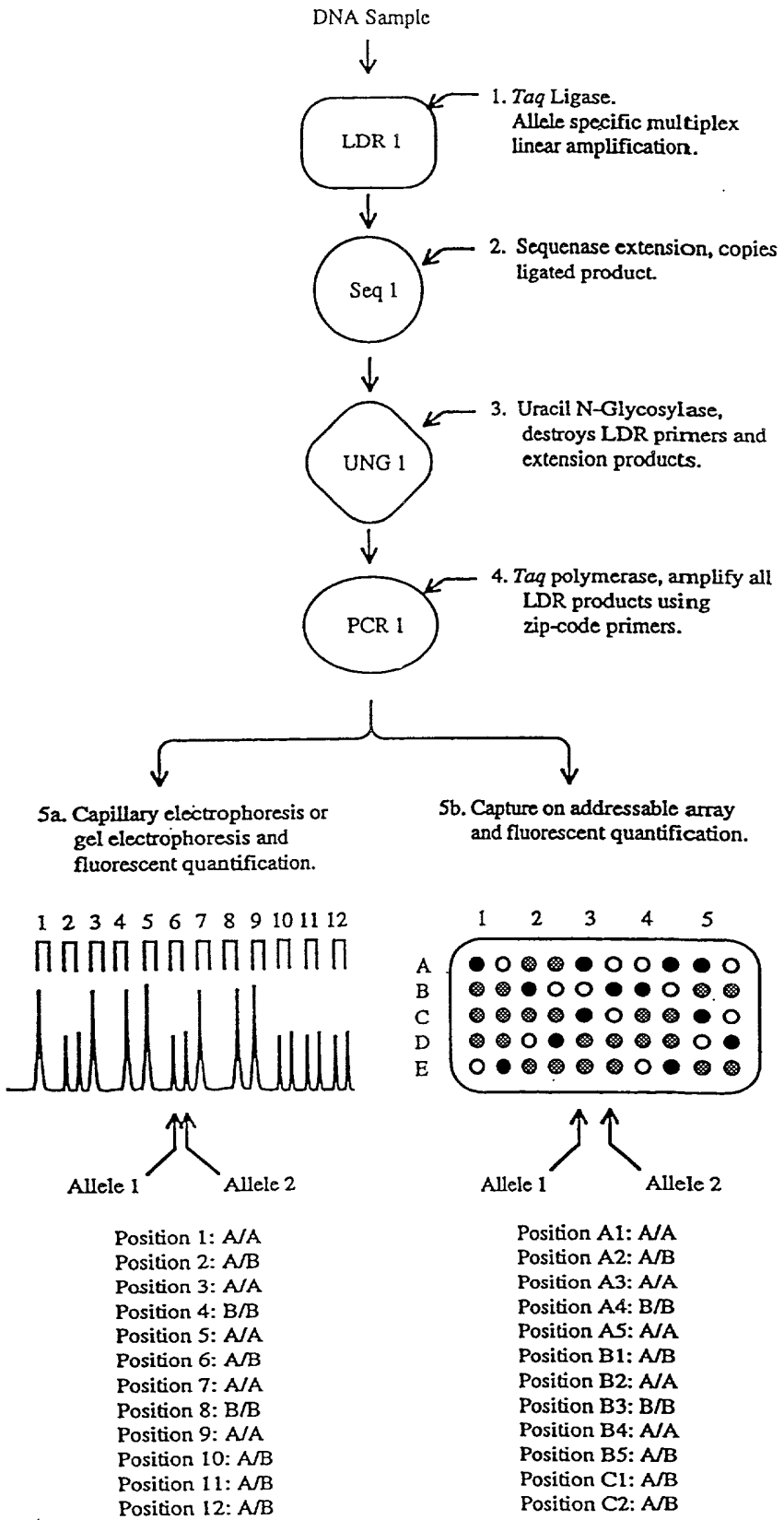

DNA Sample
LDR 1
1. *Taq* Ligase. Allele specific multiplex linear amplification.
Seq 1
2. Sequenase extension, copies ligated product.
UNG 1
3. Uracil N-Glycosylase, destroys LDR primers and extension products.
PCR 1
4. *Taq* polymerase, amplify all LDR products using zip-code primers.
5a. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
5b. Capture on addressable array and fluorescent quantification.
1 2 3 4 5 6 7 8 9 10 11 12
Allele 1
Allele 2
Position 1: A/A
Position 2: A/B
Position 3: A/A
Position 4: B/B
Position 5: A/A
Position 6: A/B
Position 7: A/A
Position 8: B/B
Position 9: A/A
Position 10: A/B
Position 11: A/B
Position 12: A/B
1 2 3 4 5
A
B
C
D
E
Allele 1
Allele 2
Position A1: A/A
Position A2: A/B
Position A3: A/A
Position A4: B/B
Position A5: A/A
Position B1: A/B
Position B2: A/A
Position B3: B/B
Position B4: A/A
Position B5: A/B
Position C1: A/B
Position C2: A/B

FIG. 18

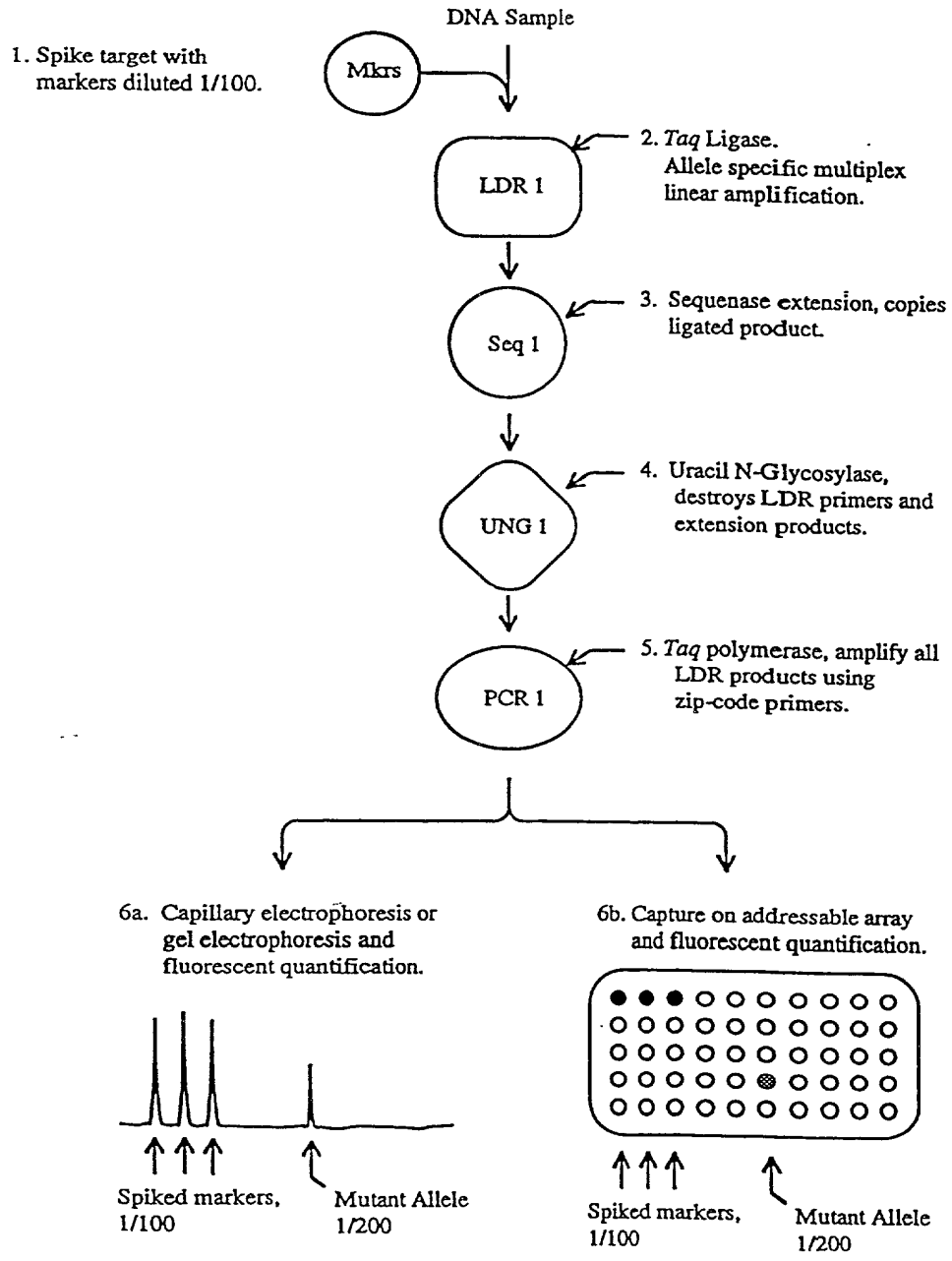

1. Spike target with markers diluted 1/100.
DNA Sample
Mkrs
LDR 1
2. *Taq* Ligase. Allele specific multiplex linear amplification.
Seq 1
3. Sequenase extension, copies ligated product.
UNG 1
4. Uracil N-Glycosylase, destroys LDR primers and extension products.
PCR 1
5. *Taq* polymerase, amplify all LDR products using zip-code primers.
6a. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
Spiked markers, 1/100
Mutant Allele 1/200
6b. Capture on addressable array and fluorescent quantification.
Spiked markers, 1/100
Mutant Allele 1/200

FIG. 19

LDR / PCR of mononucleotide repeats using Uracil N-glycosylase selection

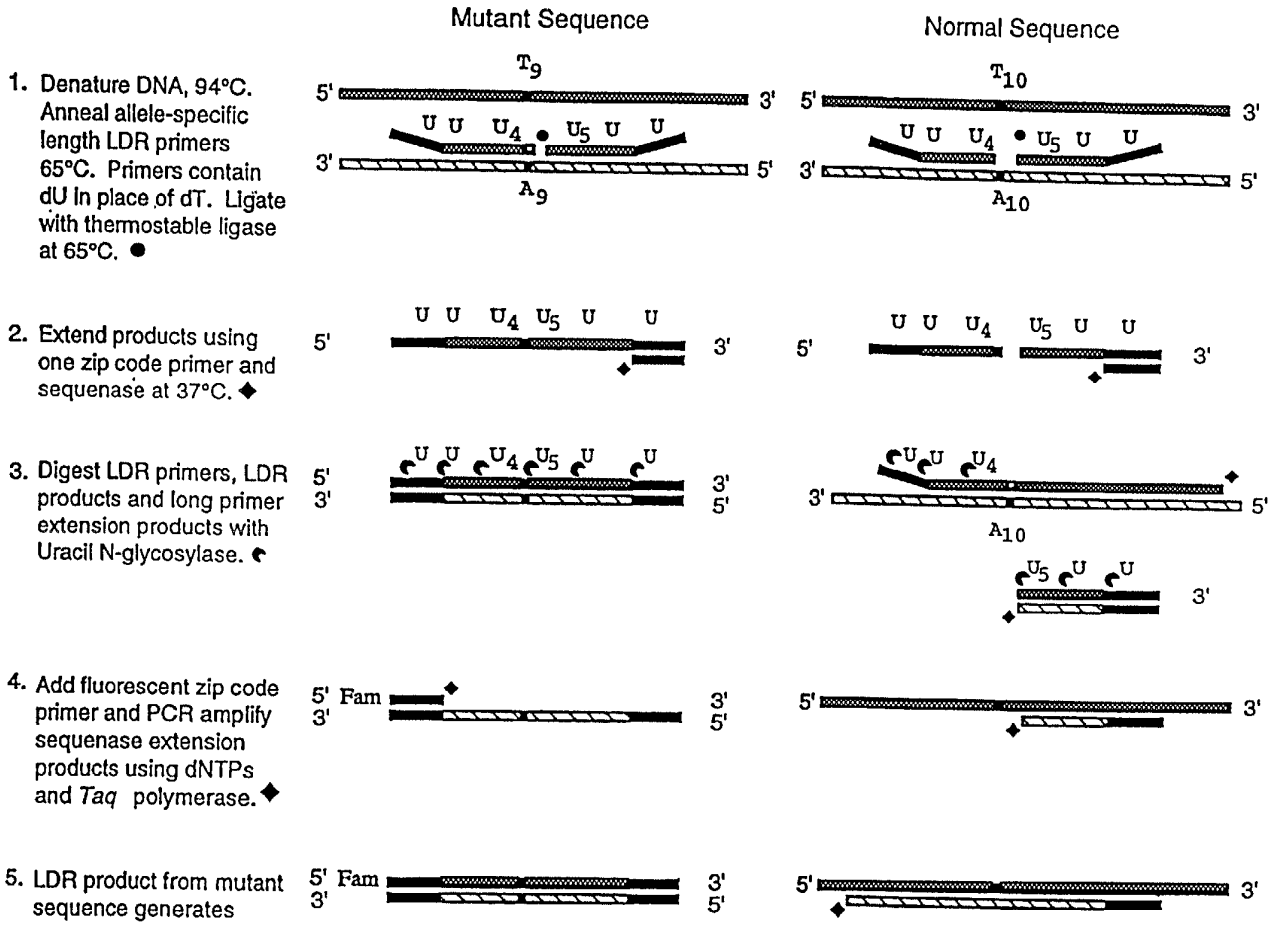


FIG. 20

LDR / PCR of mononucleotide repeat polymorphisms using Uracil N-glycosylase selection

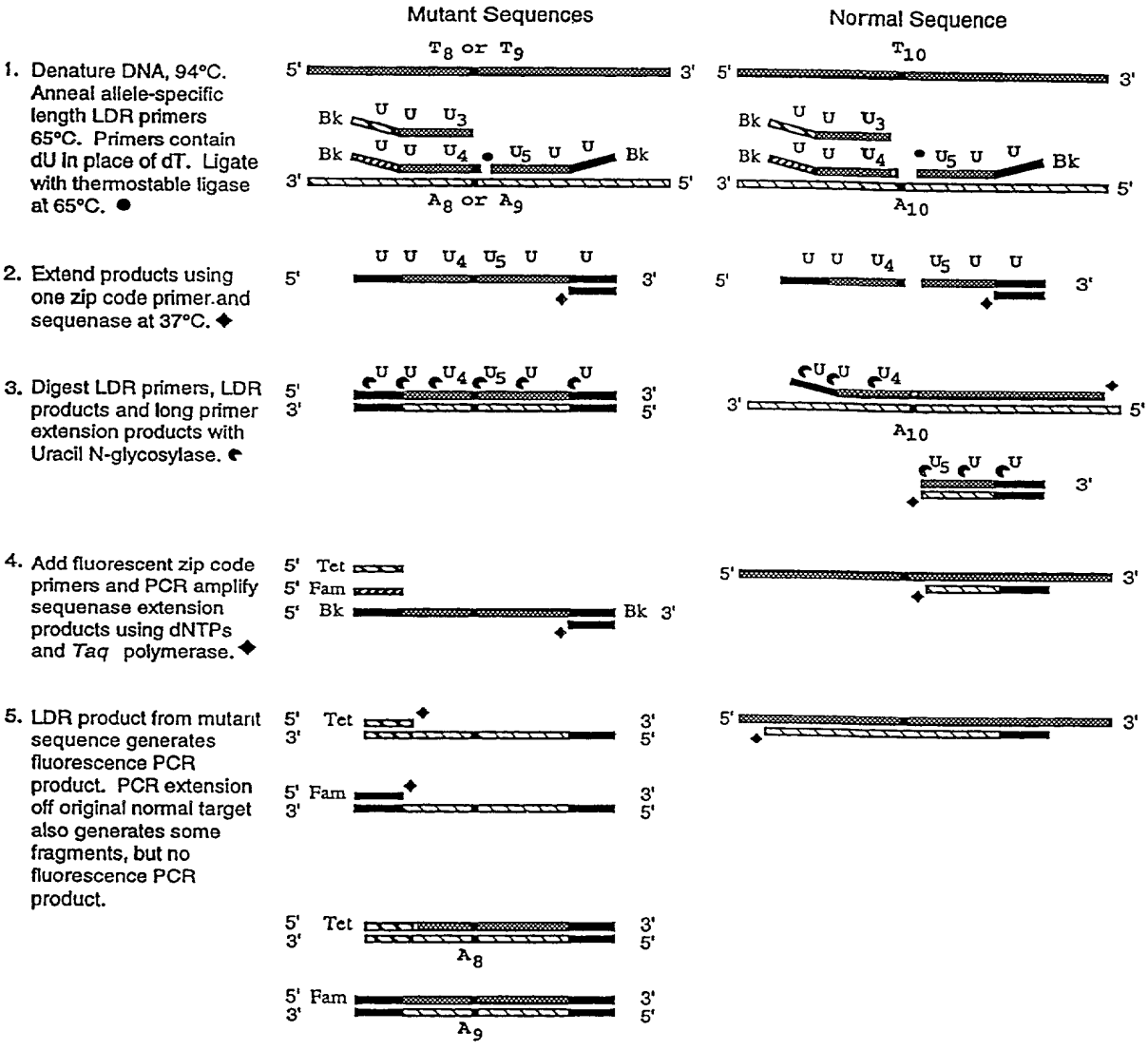


FIG. 21

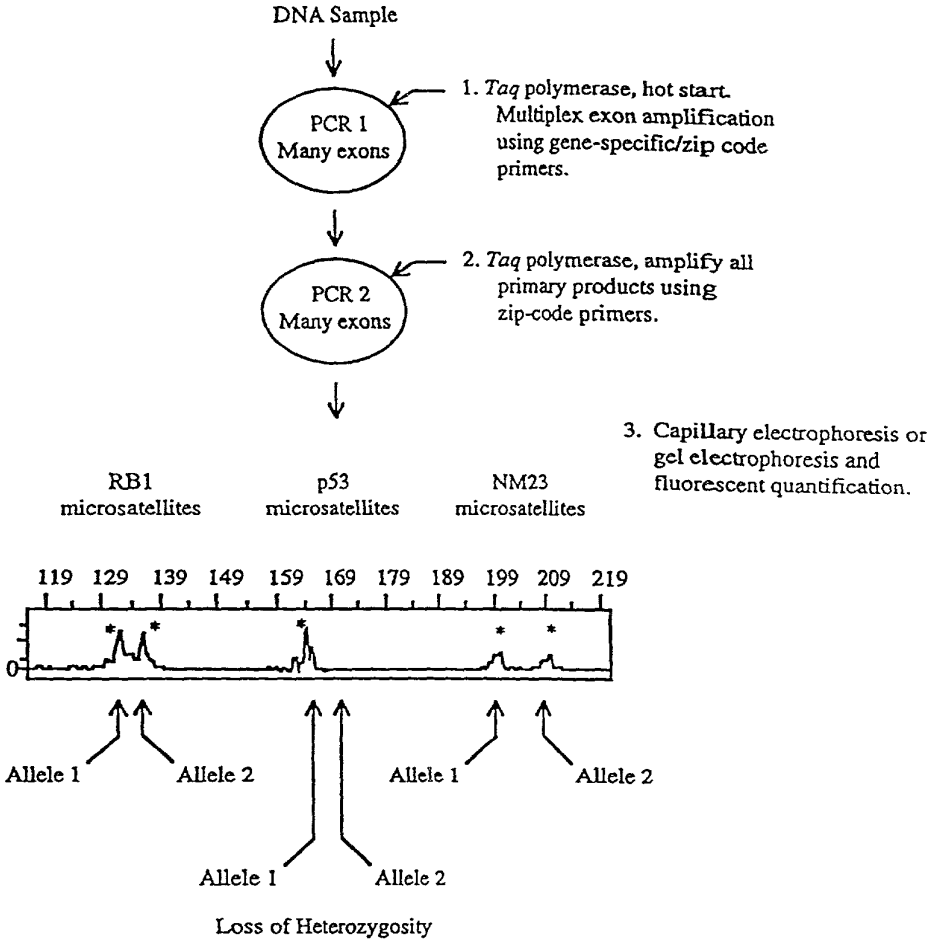

DNA Sample
PCR 1
Many exons
1. Taq polymerase, hot start. Multiplex exon amplification using gene-specific/zip code primers.
PCR 2
Many exons
2. Taq polymerase, amplify all primary products using zip-code primers.
3. Capillary electrophoresis or gel electrophoresis and fluorescent quantification.
RB1 microsatellites
p53 microsatellites
NM23 microsatellites
119 129 139 149 159 169 179 189 199 209 219
0
Allele 1
Allele 2
Allele 1
Allele 2
Allele 1
Allele 2
Loss of Heterozygosity

FIG. 22

PCR / PCR : Multiplex Microsatellite assays

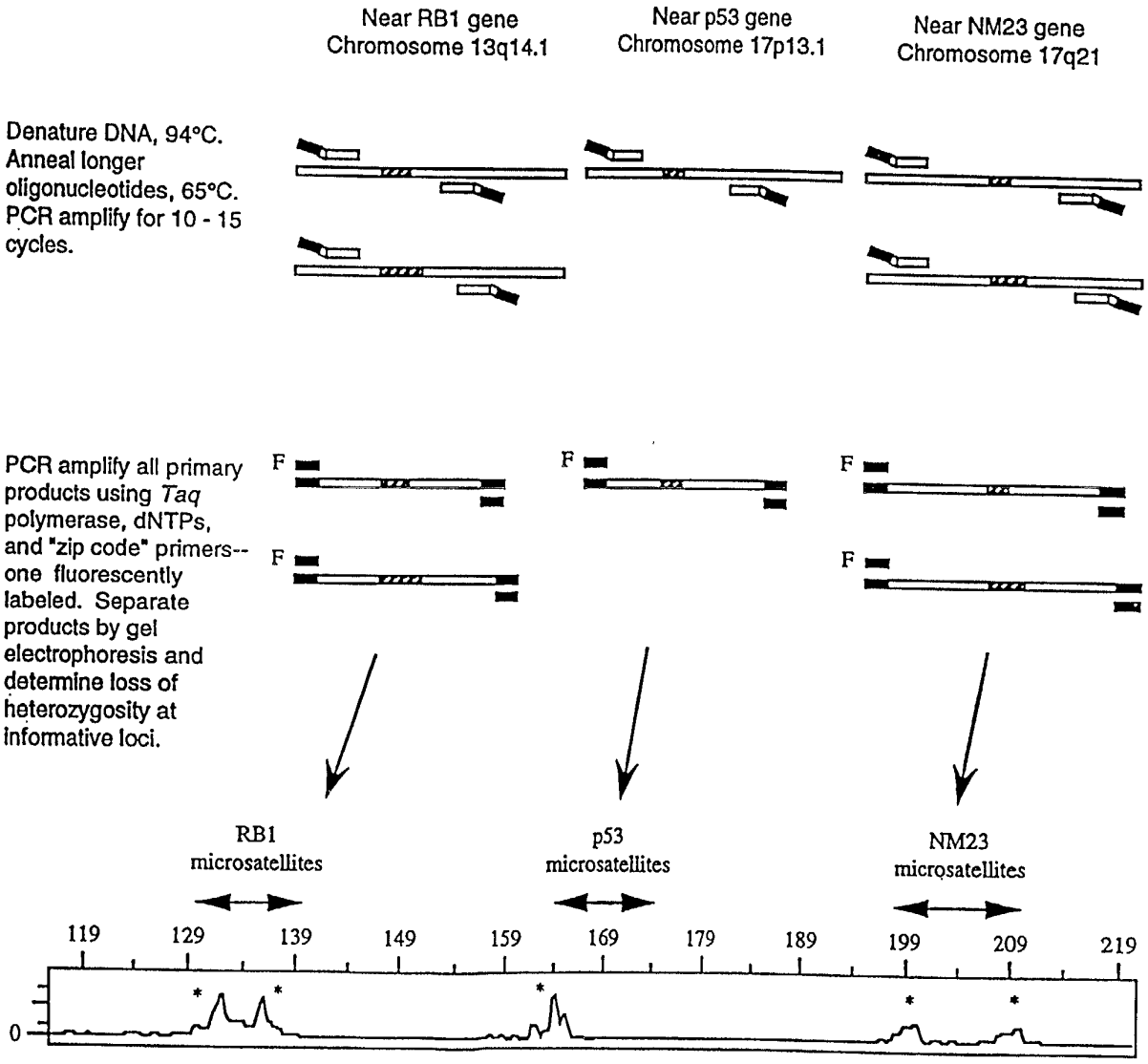


FIG. 23

Primer design for multiplex LDR / PCR

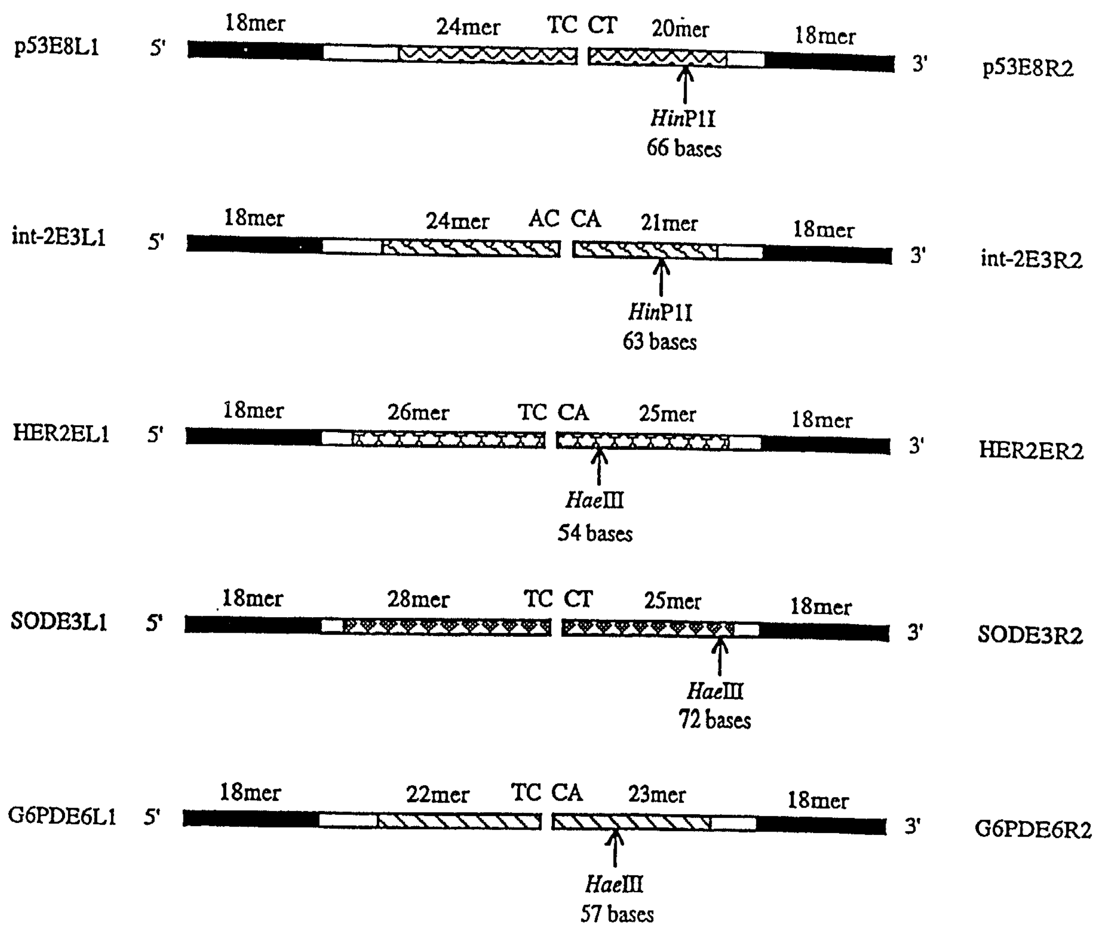


FIG. 24

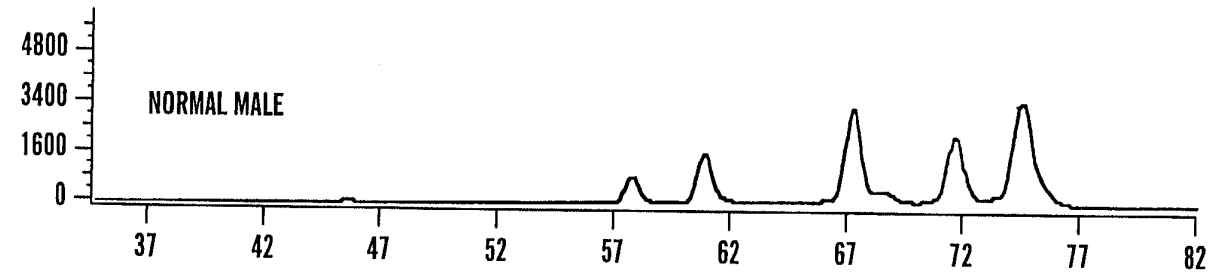


FIG. 25A

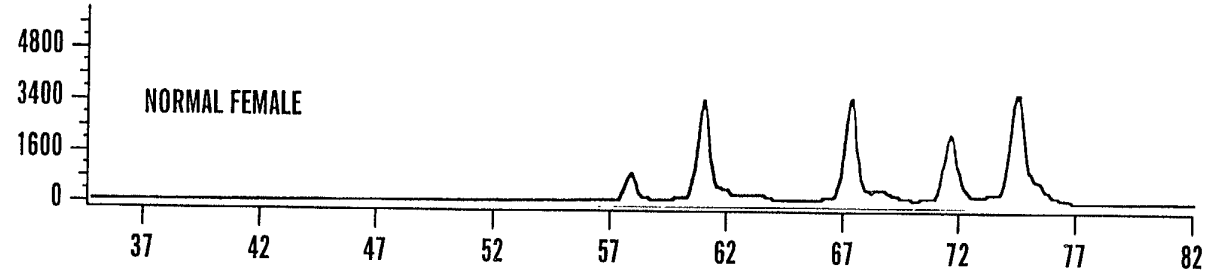


FIG. 25B

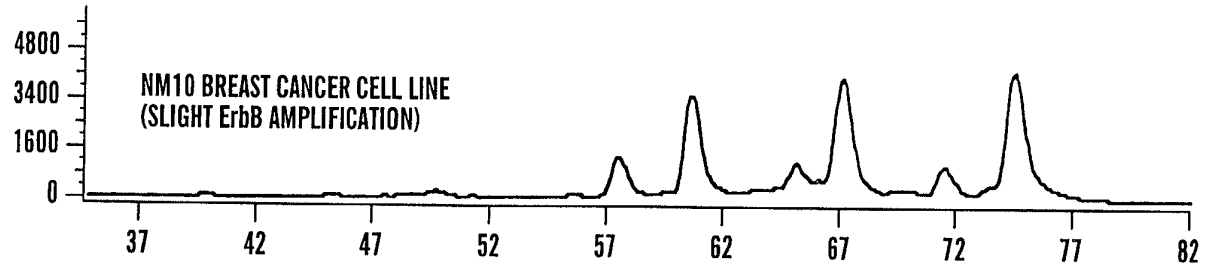


FIG. 25C

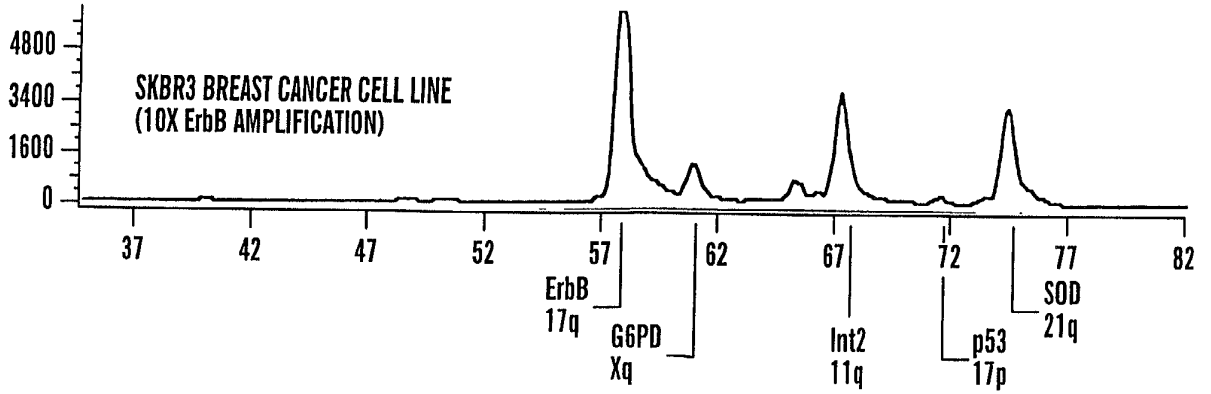


FIG. 25D

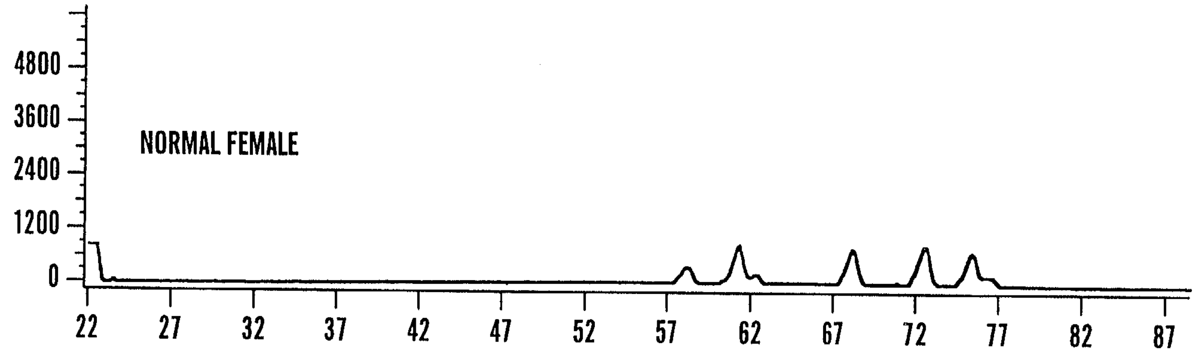


FIG. 26A

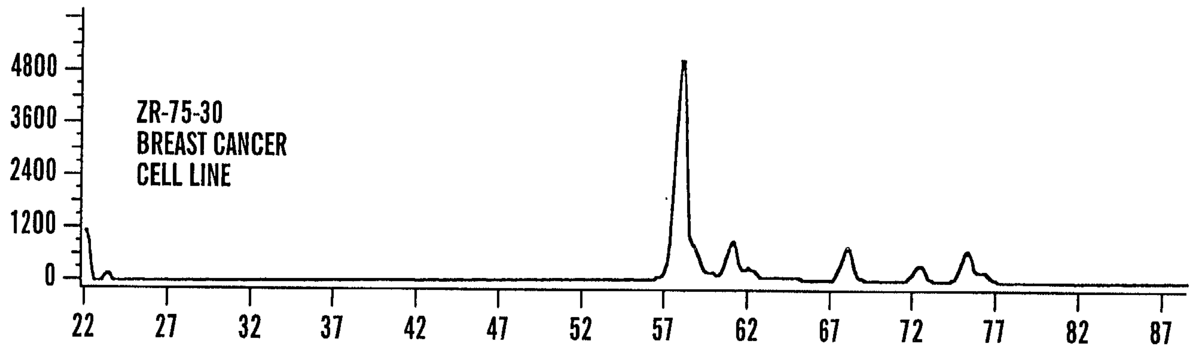


FIG. 26B

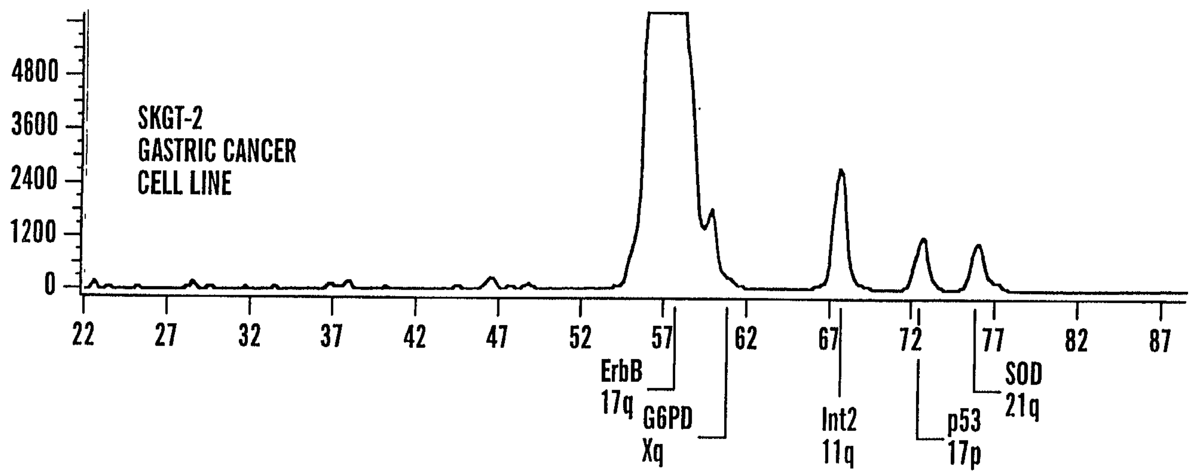


FIG. 26C

FIG. 27A

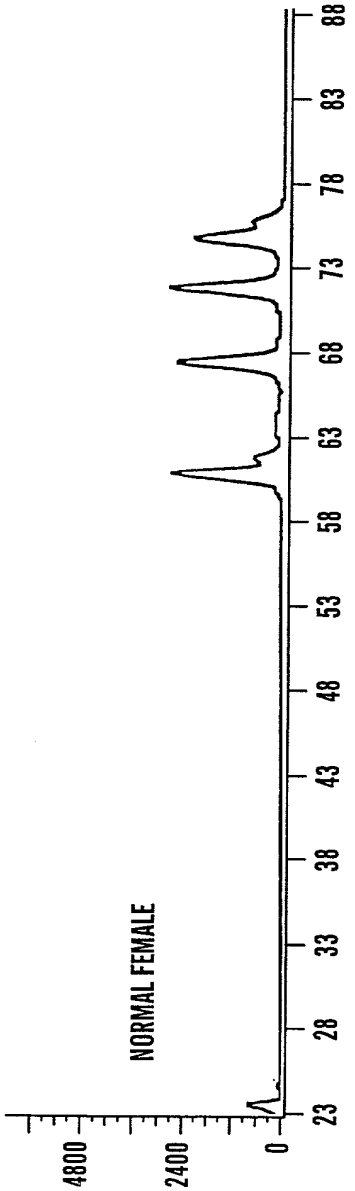
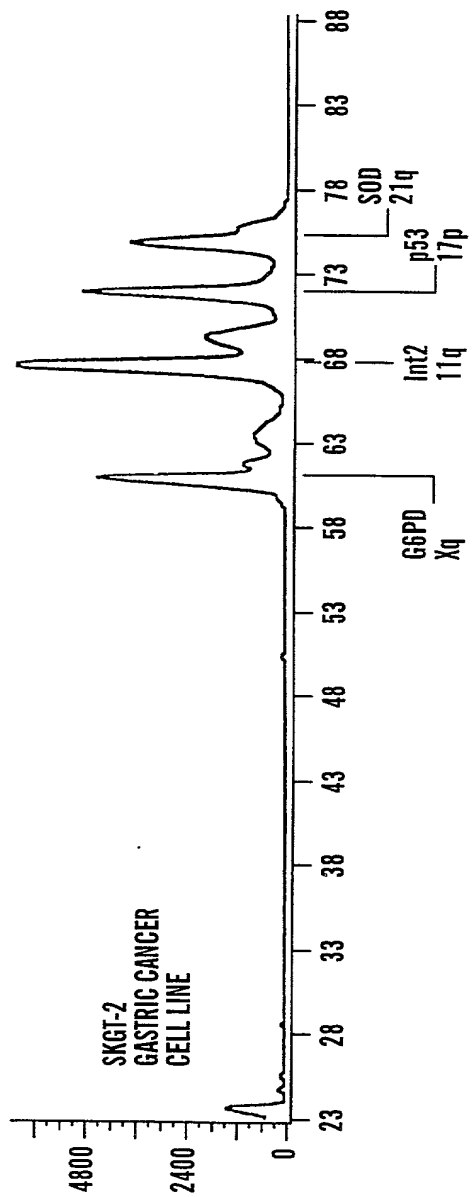


FIG. 27B

FIG. 27C



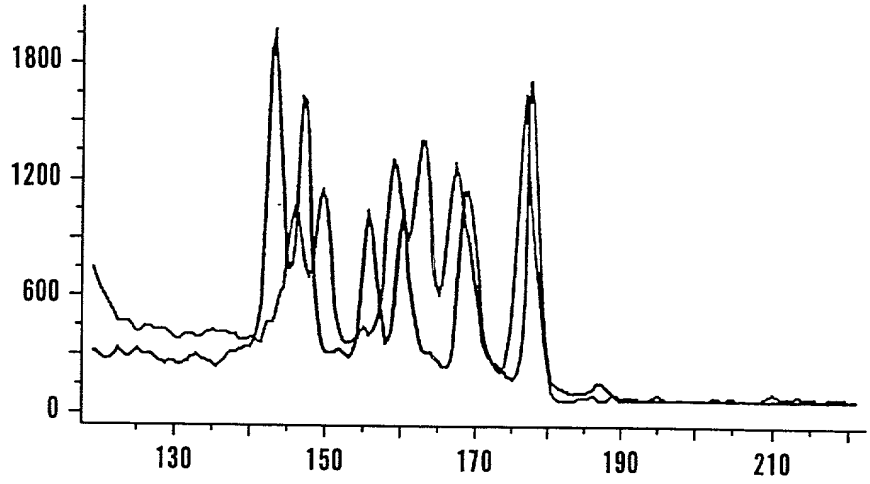


FIG. 28A

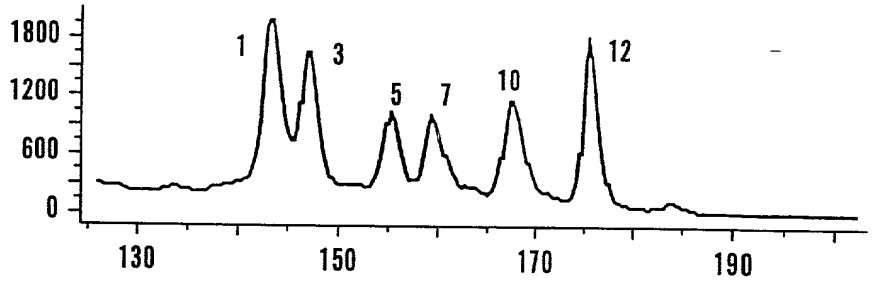


FIG. 28B

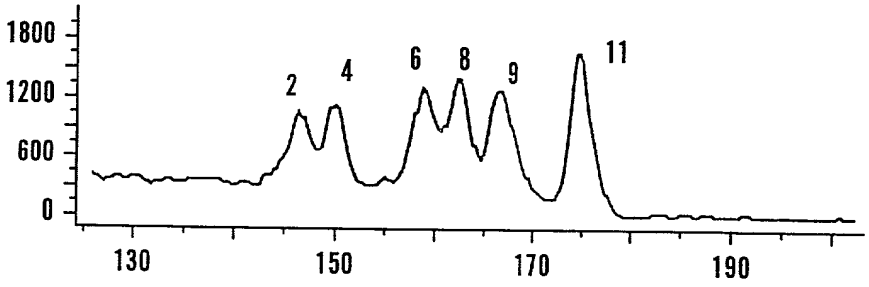


FIG. 28C

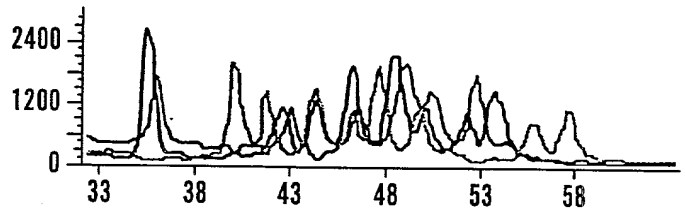


FIG. 29A

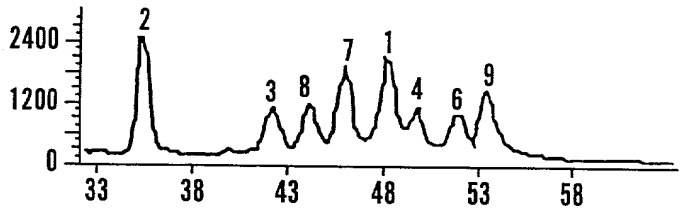


FIG. 29B

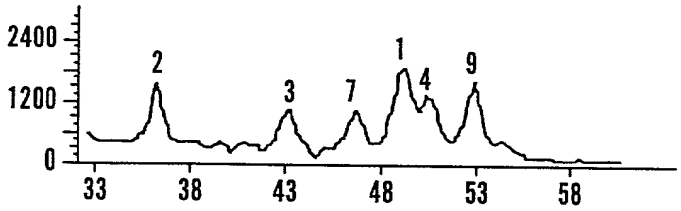


FIG. 29C

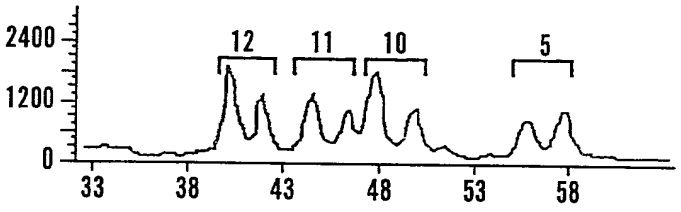


FIG. 29D

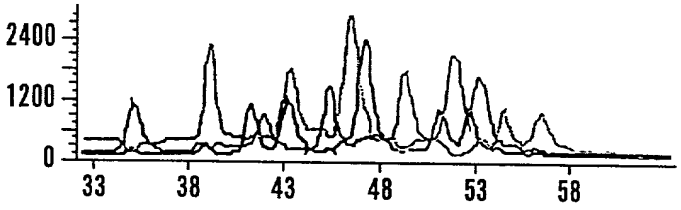


FIG. 29E

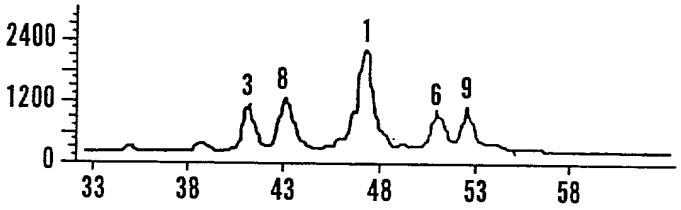


FIG. 29F

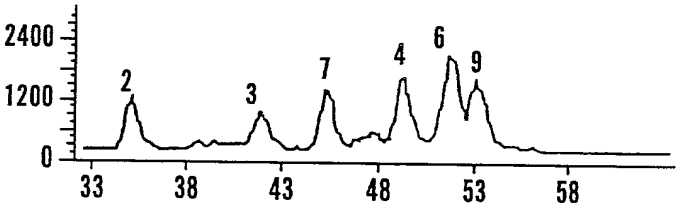


FIG. 29G

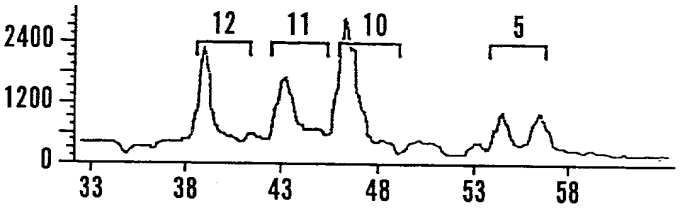


FIG. 29H